Am J Physiol Regul Integr Comp Physiol 297: R1332–R1342, 2009.
First published September 2, 2009; doi:10.1152/ajpregu.00272.2009.

Insulin-like growth factor-I and genetic effects on indexes of protein degradation in response to feed deprivation in rainbow trout (*Oncorhynchus mykiss*)

Beth M. Cleveland,[1] Gregory M. Weber,[1] Kenneth P. Blemings,[2] and Jeffrey T. Silverstein[1,3]

[1]*National Center for Cool and Cold Water Aquaculture, Agricultural Research Service-US Department of Agriculture, Kearneysville, West Virginia;* [2]*Department of Animal and Nutritional Sciences, West Virginia University, Morgantown, West Virginia; and* [3]*Office of National Programs, Agricultural Research Service-US Department of Agriculture, Beltsville, Maryland*

Submitted 18 May 2009; accepted in final form 27 August 2009

Cleveland BM, Weber GM, Blemings KP, Silverstein JT. Insulin-like growth factor-I and genetic effects on indexes of protein degradation in response to feed deprivation in rainbow trout (*Oncorhynchus mykiss*). *Am J Physiol Regul Integr Comp Physiol* 297: R1332–R1342, 2009. First published September 2, 2009; doi:10.1152/ajpregu.00272.2009.—This study determined the effect of genetic variation, feed deprivation, and insulin-like growth factor-I (IGF-I) on weight loss, plasma IGF-I and growth hormone, and indexes of protein degradation in eight full-sibling families of rainbow trout. After 2 wk of feed deprivation, fish treated with IGF-I lost 16% less ($P < 0.05$) wet weight than untreated fish. Feed deprivation increased growth hormone ($P < 0.05$) and decreased IGF-I ($P < 0.05$), but hormone levels were not altered by IGF-I. Plasma 3-methylhistidine concentrations were not affected by IGF-I but were decreased after 2 wk ($P < 0.05$) and increased after 4 wk ($P < 0.05$) of feed deprivation. In white muscle, transcript abundance of genes in the ubiquitin-proteasome, lysosomal, and calpain- and caspase-dependent pathways were affected by feed deprivation ($P < 0.05$). IGF-I prevented the feed deprivation-induced upregulation of MAFbx (F-box) and cathepsin transcripts and reduced abundance of proteasomal mRNAs ($P < 0.05$), suggesting that reduction of protein degradation via these pathways may be partially responsible for the IGF-I-induced reduction of weight loss. Family variations in gene expression, IGF-I concentrations, and weight loss during fasting suggest genetic variation in the fasting response, with considerable impact on regulation of proteolytic pathways. These data indicate that nutrient availability, IGF-I, and genetic variation affect weight loss, in part through alterations of proteolytic pathways in rainbow trout, and that regulation of genes within these pathways is coordinated in a way that supports a similar physiological response.

proteolysis; 3-methylhistidine; F-box; growth hormone

CRUDE PROTEIN REPRESENTS ~17% of the wet weight of a rainbow trout (70); therefore, it is the largest component of the dry biomass. The protein pool is in constant flux, continually being synthesized and degraded in a process referred to as protein turnover. The ability to regulate rates of protein turnover can have a significant impact on growth, weight loss, and feed efficiency. During protein accretion, protein synthesis exceeds protein degradation (17). During feed deprivation, protein degradation exceeds protein synthesis (40, 42, 53), resulting in a net loss of protein. Protein turnover can also impact feed conversion, inasmuch as differences between rates of protein synthesis and rates of protein degradation are greater in fish that exhibit high feed efficiency than in less efficient fish (17).

Insulin-like growth factor-I (IGF-I) is a hormone that increases weight gain in salmonids (44). Similar observations in mammals are attributed to the ability of IGF-I to increase protein accretion through stimulation of protein synthesis (5, 14, 23, 75) and reduction of protein degradation (23, 50, 59). In fish, stimulatory effects of IGF-I on protein synthesis have been reported (27, 49), but the extent to which IGF-I mediates proteolytic pathways in trout is unknown. However, evidence suggests that IGF-I has a more integrative role in regulation of growth, development, and metabolism in trout than in mammals. Contrary to mammals, rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) white muscle tissues have a higher abundance of IGF-I receptor protein and mRNA than insulin receptors (28, 45, 52). IGF-I also exhibits greater specific binding (52) and stimulates greater nutrient uptake than does insulin in rainbow trout myosatellite cells (11).

In feed-deprived salmonids, the reduction of plasma IGF-I (26, 77) and changes in abundance of IGF-binding proteins (56, 67), which regulate IGF-I availability (34), likely contribute to the fasting-induced reduction of cellular anabolic processes and subsequent promotion of protein catabolism. Mice (51) and rats (3) treated with IGF-I during short-term feed deprivation exhibit less weight loss than nontreated controls, which also suggests that the reduction of plasma IGF-I may be partially responsible for the onset of muscle atrophy during feed deprivation. The ability of IGF-I to induce signal transduction through the phosphatidylinositol 3-kinase (PI3K)/Akt pathway in trout myocytes (10) suggests that the reduction of IGF-I signaling during feed deprivation may serve as a mechanism for upregulation of proteasomal ubiquitin ligases during feed deprivation in trout (65), a model that is well established in mammals (22, 62).

Regulation of protein turnover during feed deprivation occurs at the transcriptional and posttranslational levels. Microarray analysis of liver (61) and white muscle (57) transcriptomes from feed-deprived trout reveals clusters of protein synthetic and proteolytic genes that were regulated in response to fasting. Proteolysis has been shown to proceed through at least four pathways: a ubiquitin-proteasome pathway, a lysosomal cathepsin pathway, a calcium-dependent pathway involving calpains, and a pathway initiated by the apoptotic caspases. Specific proteolytic responses in white muscle include increases in the mRNA abundance of lysosomal cathepsins (57), proteasomal ubiquitin ligases (65), and calpain subunits (60).

Address for reprint requests and other correspondence: B. M. Cleveland, National Center for Cool and Cold Water Aquaculture, ARS-USDA, 11861 Leetown Rd., Kearneysville, WV 25430 (e-mail: beth.cleveland@ars.usda.gov).

Effects of fasting on expression of caspases in trout white muscle have not been established. Despite the fasting-induced increase in protein degradation in the liver (53), the mRNA abundance of proteolytic genes in this tissue is generally decreased (61), suggesting that the mechanisms responsible for the metabolic effects of feed deprivation are tissue specific and may also depend on posttranscriptional processes.

This study investigates the ability of IGF-I to affect weight loss during feed deprivation through the regulation of proteolytic genes in the white muscle of rainbow trout. Understanding how nutrient deprivation and IGF-I affect proteolytic pathways will improve our knowledge of how these pathways influence protein turnover. In addition, plasma growth hormone (GH) and IGF-I have been shown to associate with growth performance among rainbow trout families (37). The integration of full-sibling families into the experimental design will determine whether genetic variation contributes to responses to feed deprivation and IGF-I.

MATERIALS AND METHODS

Animal husbandry. All rainbow trout were bred and housed at the National Center for Cool and Cold Water Aquaculture (Leetown, WV). Fish were kept in 114-liter tanks with a water exchange rate of 1.9 l/min. Inflowing water temperature was 12.5–13.8°C. Additional oxygen was supplied by an air stone purged with compressed air. All experimental procedures and protocols adhered to Animal Care and Use Committee guidelines and were approved by the Animal Care and Use Committee of the National Center for Cool and Cold Water Aquaculture.

Experimental design. Thirty-five tanks were stocked with two fish (~1 yr old) from each of eight full-sibling rainbow trout families (16 fish per tank). Fish were tagged with passive integrated transponders for individual and family identification. Fish were fed 2% of total tank weight per day (Ziegler Gold, Ziegler Bros, Gardners, PA) during a 2-wk acclimation period. Tanks were randomly divided into five treatment groups ($n = 7$): *1*) *day 1* sampling, *2*) 14-day feed-deprived (FD), *3*) 14-day FD + saline, *4*) 14-day FD + IGF-I, and *5*) 28-day FD. All treatment groups were deprived of food 2 days before the starting weight was obtained and throughout the course of the experiment. On *day 1*, fish from the *day 1* sampling group were harvested using an overdose of tricaine methanesulfonate (MS-222, Western Chemicals, Ferndale, WA). Gastrointestinal contents from one fish per family per tank were evacuated, and the fish were frozen (−20°C) for future proximate analysis. From the second fish per family per tank, blood was drawn from the caudal vasculature into a heparinized tube, and white muscle was removed and immediately frozen in liquid nitrogen. Also on *day 1*, fish from the 28-day FD group were weighed and returned to their respective tanks. On *day 2*, initial weights were obtained from fish in the 14-day FD, 14-day FD + saline, and 14-day FD + IGF-I groups. Osmotic pumps (model 1003D, Alzet, Cupertino, CA) that released 0.9% NaCl or recombinant human IGF-I (25 $\mu g \cdot kg^{-1} \cdot day^{-1}$; National Hormone and Peptide Program, Harbor-UCLA, Torrance, CA), which has binding properties and effects similar to those of recombinant trout IGF-I in rainbow trout cell culture (12, 13), were implanted in the peritoneal cavity through a 1-cm incision made ~2 cm anterior of the pelvic fin of anesthetized fish in the 14-day FD + saline and 14-day FD + IGF-I groups. The incision was closed with surgical staples and treated with triple antibiotic ointment. Fish from the 14-day FD, 14-day FD + saline, 14-day FD + IGF-I, and 28-day FD groups were harvested with a lethal dose of MS-222, and tissue was sampled as described above. Gastrointestinal tracts were empty for the 14- and 28-day samplings; therefore, the stomach contents did not require evacuation.

Proximate analysis. The comparative slaughter technique (29, 71) was used to determine treatment effects on the loss of dry weight and body fat and protein. This technique utilized the proximate composition of fish harvested on *day 1* with the recorded initial wet weights of pit-tagged fish to estimate the initial dry weight and fat and protein content for fish before feed deprivation. These data were used to calculate the total dry weight and fat and protein loss. Initial dry weight (IDW) was calculated on an individual basis as follows: $(IW * DW_I) = IDW$, where IW is initial weight (g) and DW_I is average percent dry weight (g dry matter/g wet wt) of fish harvested on *day 1*. Final dry weight (FDW) was calculated as follows: $(FW * DW) = FDW$, where FW is final weight and DW is percent dry weight (g dry matter/g body wt). Dry weight loss (DWL) was calculated as follows: $DWL = IDW - FDW$. Percent DWL was calculated as follows: $[(IDW - FDW)/IDW] * 100$. Similar equations were used to calculate fat and protein loss.

Individual fish were cut into small pieces, frozen in liquid nitrogen, and homogenized in a blender. Homogenates were stored at −20°C until analysis. Dry matter was determined by subtraction of the sample weight difference before and after drying at 105°C for 3 h. Lipid analysis was completed with an extraction system (model XT10, Ankom, Macedom, NY) according to the manufacturer's suggested protocol. Protein content was determined using the Kjeldahl procedure (2).

3-Methylhistidine analysis. Heparinized blood was centrifuged at 5,000 *g* (4°C) for 10 min. The plasma was retained and stored at −80°C until sample analysis. For deproteinization of plasma, 100 μl of plasma were mixed with 350 μl of ice-cold methanol. Samples were vortexed and incubated on ice for 30 min and then centrifuged at 15,000 *g* (4°C). The supernatant was removed and stored at 4°C until derivatization. Samples were derivatized by modification of an existing procedure (72). Stock derivatization solutions of 0.2 M *o*-phthaldialdehyde in methanol and 2% 2-mercaptoethanol in methanol were stored at −20°C. The working derivatization solution, a mixture of 10 μl of *o*-phthaldialdehyde stock and 20 μl of mercaptoethanol stock with 1 ml of 0.1 M sodium tetraborate, was made fresh daily and stored at 4°C. For sample derivatization, 50 μl of deproteinized plasma were mixed with 50 μl of derivatization solution, the sample was incubated for 2 min at room temperature, and 20 μl were immediately injected onto a C18 column (5 μm, 4.6 × 150 mm, Acclaim 120, Dionex, Sunnyvale, CA) with a 4.3 × 10 mm guard column. A modified elution protocol (74) with an electrochemical detection protocol (82) was used. The HPLC system contained a pump (model P680, Dionex), a manual injector (Rheodyne), and an electrochemical detector (model ED50, Dionex) with a gold electrode. *Eluent A* contained 12.5 mM phosphate buffer (pH 7.0) with 5 ml of tetrahydrofuran per liter. *Eluent B* contained 12.5 mM phosphate buffer (pH 7.0), acetonitrile, and tetrahydrofuran (53:40:7 by volume). Eluents were vacuum filtered and degassed by sonication before use. The flow rate was 1.2 ml/min, and the elution gradient proceeded as follows: 10% *eluent B* at 0 min, 40% *eluent B* at 4 min, 55% *eluent B* at 14 min, 100% *eluent B* at 19 min, 100% *eluent B* at 20 min, 10% *eluent B* at 22 min, and 10% *eluent B* at 32 min. Electrochemical detection settings were as follows: 10.0 Hz data collection rate and 0.70 V direct current. A standard curve was analyzed every other day with known concentrations of 3-methylhistidine (3-MH) in 50% methanol. Inter- and intra-assay variation was <5%. 3-MH recovery, which was determined by spiking a plasma sample with a known concentration of 3-MH and measuring the recovery against a standard curve, was 85–95%.

GH and IGF-I analyses. Plasma GH concentrations were measured using previously described RIA methods (54, 66) with recombinant salmon/trout GH and anti-salmon/trout primary antiserum (GroPep, Thebarton, South Australia). Concentrations of endogenous IGF-I were determined by RIA according to previously established methods (47, 69) using recombinant trout IGF-I and anti-barramundi IGF-I antiserum (GroPep). All RIA analyses were run in triplicate. Interas-

say variation was 10.9% and 12.5% for IGF-I and GH, respectively. Plasma concentrations of recombinant human IGF-I were determined in duplicate for the 14-day FD + IGF-I group as well as eight random samples from the 14-day FD + saline group with use of a commercially available human IGF-I ELISA kit (R & D Systems, Minneapolis, MN) according to the manufacturer's protocol. The ELISA did not demonstrate cross-reactivity with recombinant rainbow trout/salmon IGF-I (GroPep) at ≤9 ng/ml.

Real-time RT-PCR. mRNA abundance was quantified using real-time RT-PCR. Tissues were frozen in liquid nitrogen immediately after collection and stored at −80°C until sample processing. For RNA isolation, 50–100 mg of tissue were homogenized in 1 ml of TRIzol (Invitrogen, Carlsbad, CA) with a 5-mm steel bead in a TissueLyzer (Qiagen, Valencia, CA), and RNA was isolated according to the manufacturer's (Invitrogen) suggested standard protocol. The RNA pellet was resuspended in nuclease-free water and digested with DNase (Promega, Madison, WI) according to the manufacturer's protocol. Residual DNase was removed by a second round of TRIzol extraction, and RNA was isolated as previously described. The RNA was quantified, and the quality was estimated using the ratio of absorbance at 260 nm to absorbance at 280 nm. RNA (2 μg) was reverse transcribed using random primers (Invitrogen) and Maloney's murine leukemia virus (Promega) according to the manufacturer's protocol. cDNA was diluted 1:4 with nuclease-free water, and 2 μl were used in a 15-μl PCR with 7.5 μl of 2× SYBR Green Master Mix (Applied Biosystems, Foster City, CA), 833 nM forward primer, and 833 nM reverse primer (Table 1). The real-time PCR was performed with a sequence detection system (model 7900, Applied Biosystems) using a two-step PCR procedure with the following protocol. Temperature was held at 50°C for 2 min and then at 95°C for 10 min, 40 PCR cycles were completed at 95°C (15 s) and 60°C (1 min), and then melt curve analysis was carried out to confirm a single PCR product per reaction. In the present study, β-actin cycle threshold (C_t) values remained stable across experimental treatments; therefore, β-actin was used as a reference gene, inasmuch as it has been utilized in previous rainbow trout feed deprivation trials (32, 43, 60). Real-time PCR data were analyzed using the efficiency-corrected relative expression method (55).

Sample preparation for enzyme assays. A 20% muscle homogenate was made using a Dounce homogenizer and ice-cold buffer (50 mM HEPES, 1 mM DTT, and 0.1 mM EDTA, pH 8.0). Samples were centrifuged for 30 min at 19,000 *g* (4°C), and the supernatant was retained. Protein concentration of the supernatant was determined using the Bradford method (Pierce, Rockford, IL) with bovine serum albumin (Pierce) as a standard. All kinetic assays were fluorometric and completed using a Wallac Victor^2 plate reader (PerkinElmer, Waltham, MA).

20S proteasome assay. Proteasome activity was determined using a 20S proteasome activity kit according to the manufacturer's instructions (Alexis Biochemicals, San Diego, CA). Briefly, 25 μg of protein were added to 1× cold supplied reaction buffer containing 0.03% SDS for a total volume of 50 μl in a well of a 96-well plate. *N*-succinyl-Leu-Leu-Val-Tyr-aminomethylcoumarin in DMSO was added to a final concentration of 10 μM. The plate was rotated in a 14°C incubator for 5 min. Fluorescence was monitored over 10 min (excitation at 380 nm and emission at 460 nm).

Calpain assay. Calpain activity was determined according to kit instructions (BioVision, Mountain View, CA). Briefly, 25 μg of protein were added to the supplied extraction buffer for a final volume of 85 μl in the well of a 96-well plate. After addition of 10 μl 10× reaction buffer and 5 μl of calpain substrate (acetyl-Leu-Leu-Tyr-aminotrifluoromethylcoumarin), the plate was rotated for 5 min at 14°C. Fluorescence was then monitored over 5 min (excitation at 400 nm and emission at 505 nm).

Caspase-3 assay. Caspase-3 activity was determined according to kit instructions (BioVision). Briefly, 25 μg of protein were added to the supplied lysis buffer for a final volume of 50 μl in the well of a 96-well plate. After addition of 50 μl of 2× reaction buffer and 5 μl of 1 mM Asp-Glu-Val-Asp-aminotrifluoromethylcoumarin substrate, the sample was rotated at 14°C for 5 min. Fluorescence was monitored over 5 min (excitation at 400 nm and emission at 505 nm).

Statistical analysis. 3-MH concentrations, percent weight loss, and gene expression data were transformed before statistical analysis. 3-MH concentrations were transformed using $\log_{10}$ to create equal variances between treatment groups. For percent dry weight, fat, and protein loss, a constant was added to all values to eliminate negative

Table 1. *Primers used for real-time RT-PCR*

Gene Product	Primer Identifier	Accession No.	Primer Sequence (5′-3′)
β-Actin	BAct*	AJ438158	GCCGGCCGCGACCTCACAGACTAC
			CGGCCGTGGTGGTGAAGCTGTAAC
Ubiquitin ligase	MAFbx	CX026010	TGCGATCAAATGGATTCAAA
			GATTGCATCATTTCCCCACT
Proteasome A	ProtA*	TC78609	GGTGTAGCGCTTCTCTTTGG
			ACTGGACAAAGGTGCCTGAT
Proteasome regulatory subunit	ProtR*	TC87921	CCGACCTCAGAGAAAAGGTG
			AGAAGAGGTACTGGCGGACA
Cathepsin D	CathD*	U90321	GCCTGTCATCACATTCAACCT
			CCACTCAGGCAGATGGTCTTA
Cathepsin L	CathL*	AF358668	TGAAGGAGAAGATGTGGATGG
			TTCCTGTCTTTGGCCATGTAG
Caspase-3A	Casp3	TC139042	TTTGGGAGTAGATTGCAGGG
			TGCACATCCACGATTTGATT
Caspase-8	Casp8	TC172513	CAGCATAGAGAAGCAAGGGG
			TGACTGAGGGGAGCTGAGTT
Caspase-9	Casp9	TC138044	TTCGAGACCTGGAGACTCGT
			GCTATGCTGCCCTTTCTCAC
Calpain microsubunit	Capn1*	AY573919	GCCAAAACATTGCCTGTTATCTTAG
			ATAGGAGGCCGTATCAAAATTCC
Calpastatin short isoform	CastS*	AY937408	ATGACAGAGCAGCTGTCCAATC
			TGTTGAAGCAACATCACTGCAA
Calpastatin long isoform	CastL*	AY937407	ACGGCACCTTTCCTTTCCATTACCA
			CGGGGGGGAGCAGGAGACTTGGT

*Previously published sequences (60, 61). MAFbx, muscle atrophy F-box/atrogin-1.

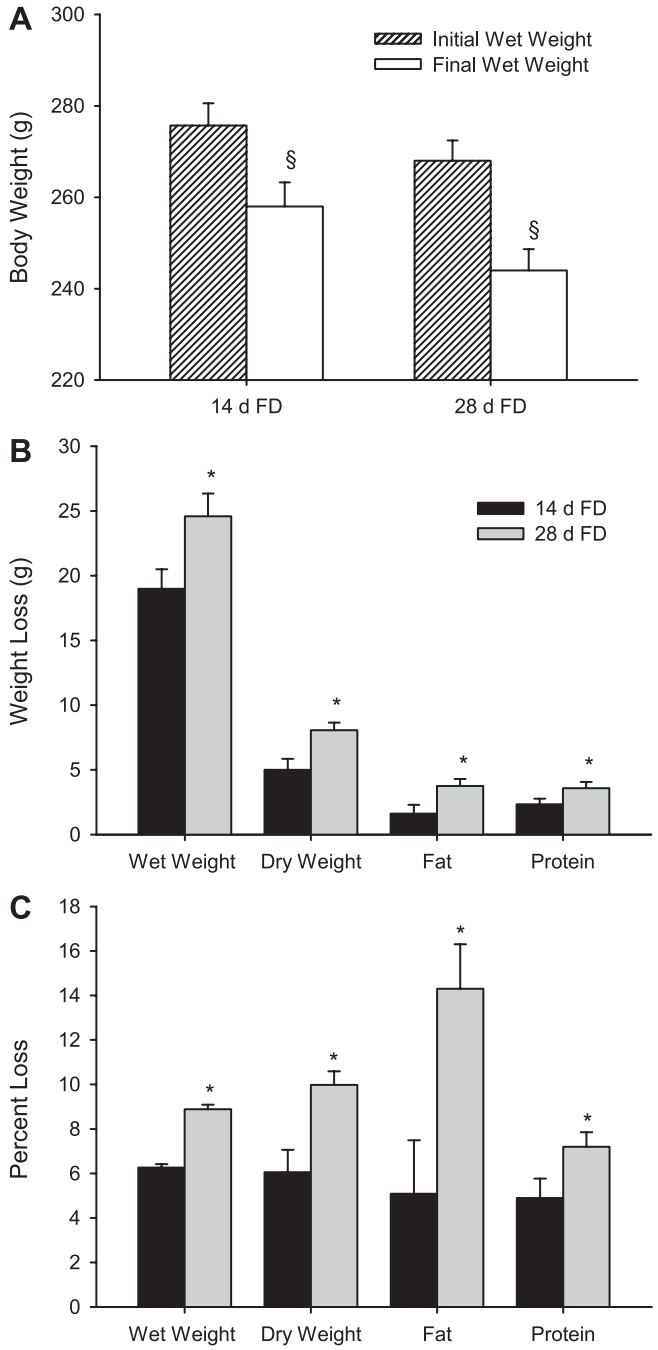

Fig. 1. Effects of 14 and 28 days of feed deprivation (14 d FD and 28 d FD) on body weight (*A*), weight loss (*B*), and percent weight loss (*C*). Values are means ± SE. §Significantly different from initial (*day 1*) body weight. *Significantly different from 14 d FD ($P < 0.05$).

percentages before data were converted to proportions. Next, the square root of the proportion and an arcsine calculation were performed before statistical analysis. Fold changes in gene expression were $\log_2$ transformed to normally distribute data. A paired *t*-test was used to detect differences in initial wet weight vs. final wet weight. ANOVA with PC-SAS (version 9.1) general linear models procedure was used to detect main effects of treatment and family and to test for treatment-by-family interactions. In the absence of a treatment-by-family interaction, independent variables were pooled to represent the main effect of treatment or family. In the event of a significant *F* value, Fisher's least significant difference procedure was used for means comparisons. For family comparisons, initial weights were used as a covariate for weight loss analysis. Interactions were tested between the covariate and treatment effect, and then they were tested for a covariate effect. Pearson product-moment correlations were used to detect correlations between percent weight loss and gene expression values. Values are means ± SE. Differences were considered significant at $P < 0.05$.

RESULTS

Weight loss. Main effects of feed deprivation on body weights are shown in Figs. 1*A* and 2*A*. As expected, fish fasted

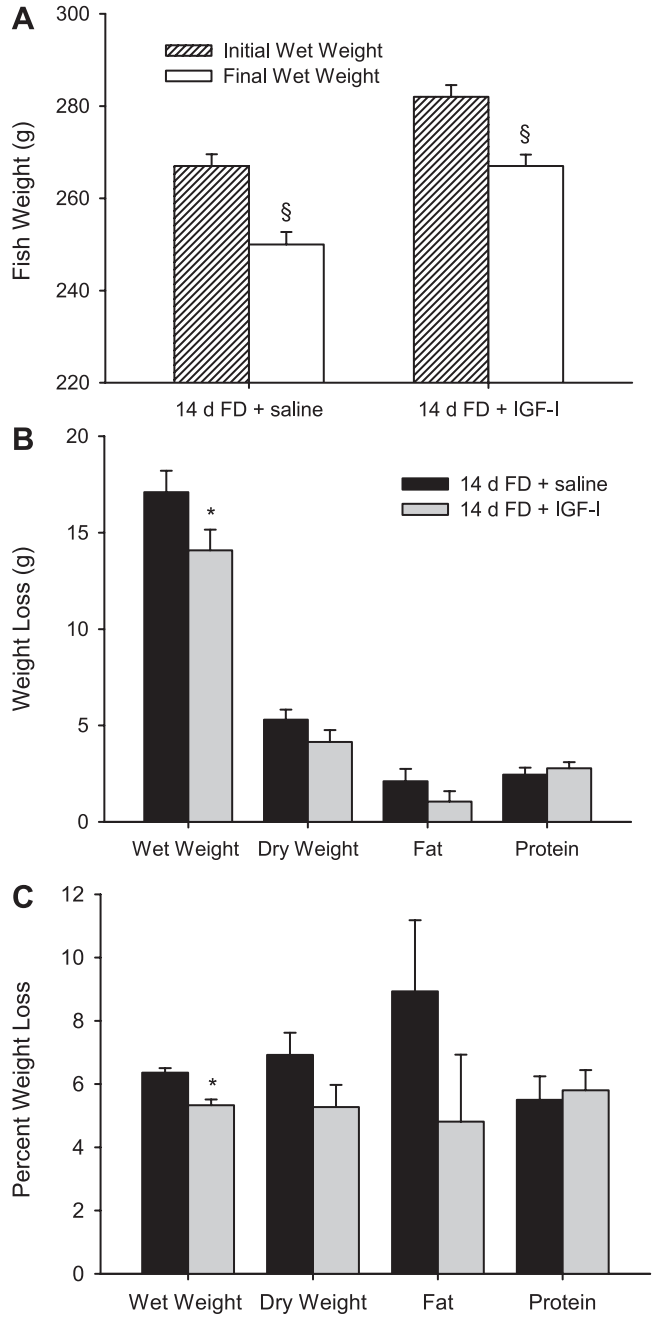

Fig. 2. Effects of insulin-like growth factor-I (IGF-I) on body weight (*A*), weight loss (*B*), and percent weight loss (*C*). Values are means ± SE. §Significantly different from initial (*day 1*) wet weight. *Significantly different from 14 d FD + saline ($P < 0.05$).

Table 2. *Family differences in initial weights and, for the 28-day FD treatment group, weight loss variables and hormone concentrations*

Family No.	Initial Wt, g (n = 70)	Wet Wt, %loss (n = 14)	Dry Wt, %loss (n = 4)	Fat, %loss (n = 4)	Crude Protein, %loss (n = 4)	GH, ng/ml (n = 5–7)	IGF-I, ng/ml (n = 6–7)
24	325±9[a]	9.1±0.7[b]	10.3±1.5	12.5±4.3	11.2±0.5	10.1±2.4	45.2±6.0[a,b]
64	209±7[g]	8.0±0.6[b]	9.1±1.5	14.2±3.9	9.1±1.9	16.8±4.0	27.2±4.4[c]
107	251±5[f]	8.9±0.4[b]	8.5±1.0	14.8±5.9	6.1±2.3	11.7±3.1	34.5±3.1[b,c]
108	288±8[b,c]	8.1±0.5[b]	10.4±0.6	10.8±4.0	8.9±2.2	12.5±1.9	52.7±2.9[a]
166	269±8[d,e]	8.2±0.4[b]	13.8±0.7	18.1±3.0	6.1±3.0	16.5±2.3	39.1±4.9[a,b,c]
167	257±7[e,f]	8.4±0.7[b]	10.5±0.7	12.6±5.4	7.3±0.8	6.3±0.6	45.1±11.1[a,b]
168	274±5[c,d]	9.4±0.4[a,b]	8.2±2.9	7.1±10.5	8.2±1.1	13.9±2.3	38.9±3.3[a,b,c]
169	293±9[b]	11.0±0.8[a]	9.3±2.8	21.1±4.7	7.9±0.5	14.9±2.7	24.8±4.9[c]

Values are means ± SE. FD, feed deprived; GH, growth hormone; IGF-I, insulin-like growth factor-I. Means within a column with superscripts without a common letter differ ($P < 0.05$).

for 28 days lost significantly more wet weight, dry weight, fat, and protein than those fasted for 14 days (Fig. 1, *B* and *C*). There were no significant differences in weight loss variables between the 14-day FD group and the 14-day FD + saline group. Wet weight loss was reduced by 16% in the 14-day FD + IGF-I group compared with the 14-day FD + saline group, but, despite similar trends in mean values, there were no detectable differences in dry weight or fat loss (Fig. 2, *B* and *C*). In addition, there was no effect of IGF-I on protein loss (Fig. 2, *B* and *C*). Proximate composition was not affected by feed deprivation or IGF-I treatment and, pooled across all treatments, averaged 29.5 ± 0.1, 9.7 ± 0.1, and 17.4 ± 0.05 g/100 g body wt for dry matter, fat and crude protein, respectively. The contribution of fat and protein loss to the total amount of dry weight loss was not different among any treatments (data not shown).

The main effect of family on weight loss in the 28-day FD group is shown in Table 2. Although there were differences in initial family weights, an analysis of covariance indicated that initial weight had no effect on variables of weight loss. *Family 169* lost the most wet weight but was not different from the remaining seven families with respect to dry weight, fat, or protein loss (Table 2). On an individual fish basis, there was a positive correlation between wet and dry weight loss ($P < 0.05$, $R^2 = 0.25$), wet weight and protein loss ($P < 0.05$, $R^2 = 0.17$), and wet weight and fat loss ($P < 0.05$, $R^2 = 0.10$). Despite the overall IGF-I amelioration of weight loss, four of the eight families did not exhibit reduced wet weight loss with IGF-I treatment (Fig. 3).

3-MH. 3-MH is a product of myofibrillar protein degradation. 3-MH concentration in muscle, plasma, and urine has been used as an index of muscle turnover in mammals (4, 24, 76), birds (19, 63), and fish (1, 73). 3-MH concentrations were reduced in the 14-day FD group compared with the *day 1* group (Table 3). 3-MH concentrations were higher in the 28-day FD group than in the *day 1* group. Compared with saline-treated fish, IGF-I treatment did not affect 3-MH concentrations. There was no effect of family, nor was there a family-by-treatment interaction, for 3-MH concentrations.

Plasma GH and IGF-I. Treatment effects on plasma concentrations of GH and endogenous IGF-I are shown in Fig. 4. Feed deprivation increased plasma GH, but GH was not affected by IGF-I treatment. IGF-I concentrations were decreased after 14 days of feed deprivation and were reduced further after 28 days of feed deprivation. Although IGF-I concentrations were significantly lower in the 14-day FD + saline group than in the 14-day FD group, they were not different from the 14-day FD + IGF-I group. Human IGF-I concentration in the 14-day FD + IGF-I group averaged 22.3 ± 2.5 ng/ml. Despite the absence of ELISA cross-

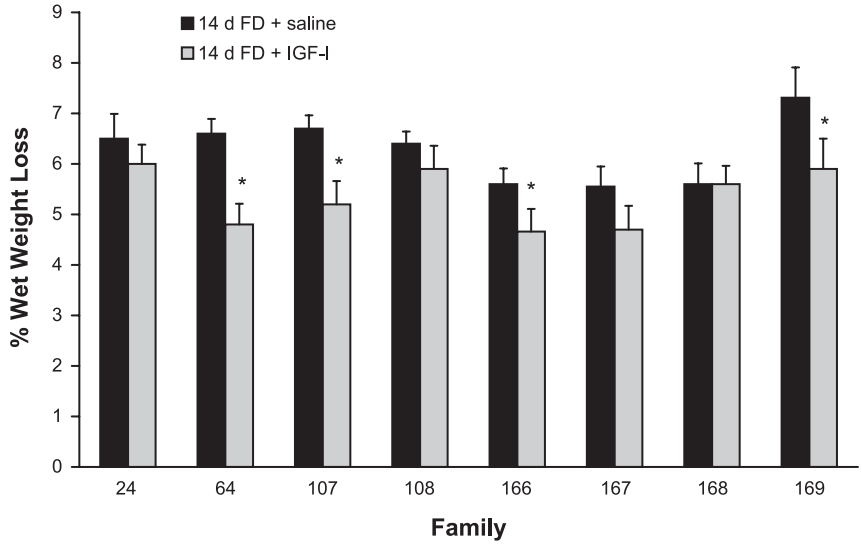

Fig. 3. Effect of IGF-I on wet weight loss for each family. Values are means ± SE. *Significantly different from 14 d FD + saline ($P < 0.05$).

Table 3. *Effect of treatment on plasma 3-MH concentrations and muscle protein activity*

Treatment Group	3-MH, μM (n = 45–51)	Muscle Protein Activity, RFU·min^{-1}·μg protein^{-1} (n = 15–16)		
		20S Proteasome	Calpain	Caspase-3
Day 1	3.8±0.3[b]	24.1±1.7[b]	12.2±0.5[a]	1.3±0.2
14-day FD	1.9±0.7[c]	33.6±5.3[a,b]	1.7±0.4[b]	1.5±0.3
14-day FD + saline	1.9±0.5[c]	36.0±4.2[a]	1.9±0.2[b]	1.8±0.2
14-day FD + IGF-I	2.1±0.4[c]	24.2±3.0[b]	1.3±0.2[b]	2.0±0.2
28-day FD	10.2±2.3[a]	31.1±2.6[a,b]	2.2±0.2[b]	1.7±0.7

Families were pooled for treatment means ± SE. 3-MH, 3-methylhistidine; RFU, relative fluorescence units. Means within a column with superscripts without a common letter differ ($P < 0.05$).

Table 4. *Main effect of feed deprivation on percent change in mRNA abundance from fish harvested on day 1*

Gene Product	14-day FD	28-day FD
Proteasome		
MAFbx	532±69†	1,216±225*†
Proteasome A	−9.5±8.3	25.1±11.7*
Proteasome R	−24.4±9.9†	8.9±12.0*
Cathepsins		
Cathepsin D	44.9±13.7†	72.5±14.7†
Cathepsin L	46.4±9.2†	77.4±17.8†
Caspases		
Caspase-3	−28.4±7.1†	−34.7±5.7†
Caspase-8	−18.8±4.2†	11.8±8.1*
Caspase-9	−4.7±4.0	16.5±8.2
Calpains		
Calpain 1	−30.8±5.3†	−36.5±18.7†
Calpastatin S	69.4±18.2†	104.1±25.7†
Calpastatin L	1.9±4.8	98.0±31.3*†

Families were pooled for treatment means ± SE; n = 48 for each gene product. *Significant difference from 14-day FD ($P < 0.05$). †Significant difference from fish harvested on *day 1* ($P < 0.05$).

reactivity with recombinant trout IGF-I, the background reading of plasma from the 14-day FD + saline group was equivalent to 10.1 ± 0.6 ng/ml human IGF-I. Therefore, the actual human IGF-I concentration in the 14-day FD + IGF-I group may be lower than the reported 22.3 ng/ml. The main effect of family on IGF-I concentrations in the 28-day FD group is shown in Table 2. Within this data set, there were no correlations between family averages of plasma GH and IGF-I or between hormone concentrations and weight loss variables. However, on an individual basis, there was a negative correlation between plasma IGF-I and percent wet weight loss in the 14-day FD ($P < 0.001$, $R^2 = 0.28$) and 28-day FD ($P < 0.001$, $R^2 = 0.42$) groups.

Gene expression. Treatment effects on expression of proteolytic genes in white muscle are shown in Table 4. Feed deprivation affected mRNA abundance of transcripts involved in the four proteolytic pathways. In the ubiquitin-proteasome pathway, the abundance of muscle atrophy F-box/atrogin-1 (MAFbx) mRNA increased 532% and 1,216% in fish fasted for 14 and 28 days, respectively. Proteasome subunit R mRNA exhibited transient downregulation only after 14 days without feed. Cathepsin mRNA abundance was similar between the short and long isoforms, with a 45–75% increase in expression during feed deprivation. Feed deprivation-induced regulation of caspase expression varied among caspase transcripts. Abundance of caspase-3 mRNA was decreased ~30% across both time periods, and caspase-8 mRNA exhibited a transient 19% reduction after 14 days of fasting. Components of the calpain pathway were also differentially regulated by feed deprivation, with the mRNA abundance of the calpain microsubunit decreasing by 30–37%. During fasting, transcript abundance of the calpastatin short isoform increased by 69–104%. Expression of the calpastatin long isoform mRNA was increased 98% only after 28 days of feed deprivation. Together, these data suggest that, in white muscle, transcriptional regulation of proteolytic genes explains, in part, changes in protein degradation during periods of fasting.

The strongest effects of IGF-I on expression of proteolytic genes in white muscle were in the ubiquitin-proteasome and cathepsin pathways (Table 5). Treatment with IGF-I reduced the upregulation of MAFbx and reduced the expression of proteasome subunit A and R mRNAs. IGF-I prevented the increase in transcript abundance for cathepsins D and L. In addition, IGF-I slightly reduced the downregulation of caspase-8 expression.

The main effect of family on percent change in mRNA abundance after 28 days of feed deprivation is shown in Table 6. Signif-

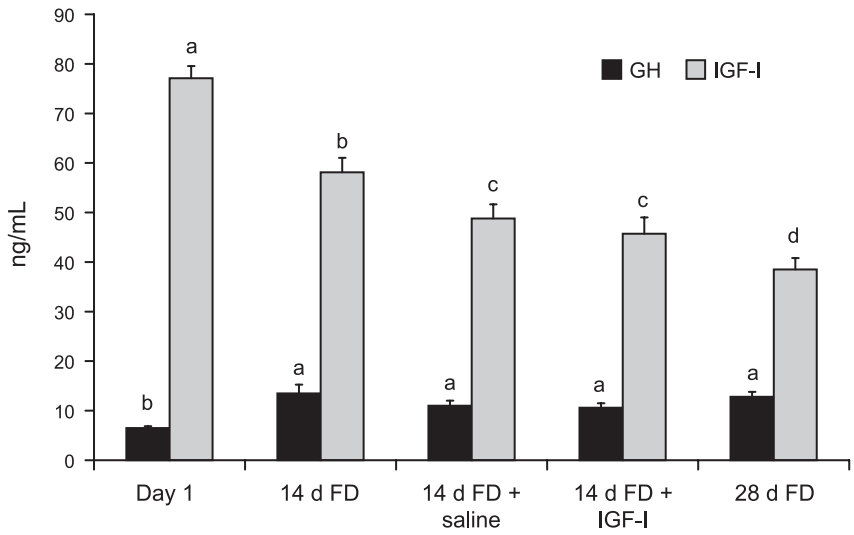

Fig. 4. Main effect of treatment on plasma concentrations of growth hormone (GH) and IGF-I. Values are means ± SE. Means with superscripts without a common letter differ ($P < 0.05$).

Table 5. *Main effect of feed deprivation and IGF-I on percent change in mRNA abundance from fish harvested on day 1*

Gene Product	14-day FD + Saline	14-day FD + IGF-I
Proteasome		
MAFbx	536±97†	185±60*†
Proteasome A	2.2±6.8	−34.9±8.5*†
Proteasome R	−1.9±12.3	−59.4±3.5*†
Cathepsins		
Cathepsin D	52.7±8.2†	7.9±10.2*
Cathepsin L	75.3±7.5†	−7.7±11.1*
Caspases		
Caspase-3	−31.6±3.6†	−33.8±3.1†
Caspase-8	−27.7±3.9†	−13.1±3.6*
Caspase-9	−2.8±4.4	0.1±4.2
Calpains		
Calpain 1	−8.9±10.7	−14.2±9.0
Calpastatin S	63.9±12.7†	46.7±12.5†
Calpastatin L	21.7±7.6†	8.2±9.5

Values are means ± SE; $n = 48$ across treatments for each gene product. *Significant difference from 14-day FD + saline ($P < 0.05$). †Significant difference from fish harvested on *day 1* ($P < 0.05$).

icant differences between families were detected for MAFbx, proteasome subunit A, and the calpastatin long isoform, supporting the presence of genetic variation in the fasting response.

Correlations between family means for percent change in gene expression and weight loss after 28 days of feed deprivation are shown in Table 7. Correlations were observed between family wet weight loss and changes in gene expression only in families subjected to 28 days of feed deprivation. Data for proteasome subunit R, cathepsin L, and caspase-9 indicate that families exhibiting higher gene expression values also demonstrate a greater weight loss after 28 days of feed deprivation (Table 7). Similar trends ($0.05 < P < 0.10$) occur for proteasome subunit A, cathepsin D, and cathepsin L expression. Correlations between gene expression and weight loss were not observed after 14 days of feed deprivation or during treatment with IGF-I or saline (data not shown). In addition, correlations between genes within and among proteolytic pathways were observed after 14 days (data not shown) and 28 days (Table 7) of fasting. The majority of these correlations were observed between genes in the ubiquitin-proteasome and cathepsin pathways, as well as with caspase-8. Together, these data suggest that regulation of these pathways is integrated in a way that supports a coordinated physiological response to feed deprivation in rainbow trout. However, a potential caveat to this conclusion occurs when Bonferroni corrections are applied for the correlations in Table 7; then only $P \leq 0.0011$ is significant. With this correction, only the correlation between proteasome A and cathepsin L remains significant, which potentially alters the impact of our findings. There were no correlations between family averages of GH or IGF-I and gene expression levels (data not shown). However, for individual fish within the 28-day FD group, there were negative correlations between plasma IGF-I and MAFbx expression ($P = 0.048$, $R^2 = 0.10$) and plasma IGF-I and proteasome R expression ($P = 0.044$, $R^2 = 0.15$).

Proteolytic activity. Treatment effects on proteolytic activities in white muscle are shown in Table 3. Activity of the proteasome increased in fish treated with saline, but not in fish treated with IGF-I. Feed deprivation decreased calpain activity but did not affect caspase-3 activity. No family effects were observed for any proteolytic activities.

DISCUSSION

Wet and dry weight loss data indicate that, across all families, fish lost more weight between *days 1* and *14* of fasting than between *days 15* and *28* of fasting. Metabolic rates decrease during feed deprivation in fish (33, 42); therefore, the reduction in metabolic rate, and consequently the caloric requirement, would result in reduced nutrient mobility and lead to a reduction in the rate of weight loss. This concept is supported by a previous observation in rainbow trout of a progressive reduction in the rate of weight loss throughout a 56-day fasting period (40). The percentage of fat lost during the second 2-wk period of fasting nearly doubled compared with that during the first 2-wk period, suggesting that, as previous studies support (35, 58), glycogen stores, hepatic lipids, and perivisceral fat are utilized in preferential order as energy substrates during feed deprivation in fish.

In fasting rainbow trout white muscle, the fractional rate of protein degradation increases during the first 14 days and decreases thereafter (40). 3-MH data in the present experiment suggest that myofibrillar degradation was reduced during the first 2-wk period of fasting and increased during the second 2-wk period. In the aforementioned study (40), the fractional rate of protein degradation was calculated using protein synthesis rates determined by radiotracer methodology and the specific growth rate. This approach assumes that the overall change in body weight approximates white muscle loss, but it is more likely that utilization of nutrient stores during feed deprivation occurs in a preferential manner, with the consumption of perivisceral fat and glycogen stores preceding mobilization of protein. In this case, the assumption would force the overestimation of white muscle fractional rates of protein degradation, which may contribute to the discrepancy between the present data and previously published data (40).

Consistent with previous research (9, 26, 77), feed deprivation increased plasma concentrations of GH and reduced plasma concentrations of IGF-I. In rainbow trout, IGF-I is known to inhibit GH release from the pituitary (7); therefore, the reduction of IGF-I during feed deprivation likely contributes to the elevation of plasma GH. Although treatment with IGF-I did not significantly reduce GH concentrations below the level observed after saline treatment, IGF-I-treated fish exhib-

Table 6. *Main effect of family on percent change in transcript abundance after 28 days of feed deprivation*

	Primer Identifier		
Family No.	MAFbx	Proteasome A	Calpastatin L
24	2,890±784[a]	113.6±47.4[a]	260.0±118.0[a]
64	1,041±335[a,b]	19.2±19.0[a,b,c]	5.7±9.6[a,b]
107	965±200[a,b]	−11.1±11.5[b,c]	173.3±57.4[a]
108	313±128[c]	−24.2±15.2[c]	46.6±68.2[a,b]
166	850±295[a,b,c]	−13.0±10.5[c]	29.3±33.0[a,b]
167	771±582[b,c]	−13.0±32.0[c]	−30.6±28.3[b]
168	811±100[a,b,c]	41.7±24.2[a,b,c]	25.9±18.2[a,b]
169	1,653±902[a,b]	81.5±44.0[a]	282.7±138.0[a]

Values (percent change in transcript abundance from *day 1*) are means ± SE; $n = 6$ across families for each primer identifier. Means within a column with superscripts without a common letter differ ($P < 0.05$).

Table 7. *Pearson's correlation and P values between family means of 28-day FD wet weight loss and percent change in gene expression*

	Gene Product								
	MAFbx	Proteasome A	Proteasome R	Cathepsin D	Cathepsin L	Caspase-8	Caspase-9	Calpastatin L	Calpastatin S
%Loss									
ρ	0.513	0.680	0.722	0.686	0.838	0.607	0.883	0.679	−0.070
P	0.193	0.064	**0.043**	0.060	**0.009**	0.111	**0.004**	0.064	0.870
MAFbx									
ρ		0.857	0.874	0.871	0.609	0.660	0.653	0.626	0.197
P		**0.007**	**0.005**	**0.005**	0.109	0.747	0.079	0.097	0.639
Proteasome A									
ρ			0.869	0.994	0.719	0.670	0.852	0.616	0.265
P			**0.005**	**0.001**	**0.044**	0.691	**0.007**	0.103	0.525
Proteasome R									
ρ				0.901	0.848	0.858	0.746	0.587	0.205
P				**0.002**	**0.008**	**0.006**	**0.033**	0.126	0.626
Cathepsin D									
ρ					0.768	0.741	0.837	0.578	0.312
P					**0.026**	**0.035**	**0.010**	0.133	0.452
Cathepsin L									
ρ						0.888	0.684	0.498	0.127
P						**0.003**	0.612	0.210	0.764
Caspase-8									
ρ							0.478	−0.313	0.192
P							0.231	0.449	0.650
Caspase 9									
ρ								0.596	0.061
P								0.119	0.887
Calpastatin L									
ρ									0.630
P									0.094

$P < 0.05$ values are shown in boldface type.

ited the numerically lowest GH concentrations. GH release is influenced by a variety of factors (79), which may have diminished the effects of human IGF-I on GH release. The reduction of plasma IGF-I during feed deprivation is likely partially responsible for the associated increase in protein catabolism. This has been demonstrated in previous rodent studies (3, 51), but in this study the mechanisms responsible for regulation of protein catabolism were also investigated. Although IGF-I treatment did not elevate plasma IGF-I concentrations to levels comparable those in the fed state, IGF-I treatment did partially offset the feed deprivation-induced reduction of IGF-I, the effects of which were observed as differences in weight loss and expression of proteolytic genes. In salmonids, 0.3–0.4% of total IGF-I is free in solution (68), but the extent to which human IGF-I interacts with and regulates abundance of IGF-binding proteins in trout has not been characterized. These potential effects may have contributed to the amount of free IGF-I that is available for receptor binding and the subsequent regulation of gene expression in the present study.

Feed deprivation induces muscle atrophy, which is characterized by loss of muscle protein (6, 30, 33), and changes in the expression of proteolytic genes reflect regulation of the involved pathways. Evidence in mammals suggests that the ubiquitin-proteasome pathway is responsible for the majority of protein degradation during muscle atrophy (36, 38). The observed upregulation of the ubiquitin ligase MAFbx during fasting is consistent with induced muscle atrophy, as previously reported in trout (57, 65) and other mammalian models of muscle wasting (8, 62). A previous study in rainbow trout (65) suggests that the upregulation of MAFbx increases the potential for ubiquitination and protein degradation via the proteasome. In the present experiment, the increase in MAFbx expression after 2 wk of feed deprivation implies that the capacity for protein degradation is elevated during this time; however, the reduction of plasma 3-MH concentrations after the 2-wk treatment suggests that proteolysis was decreased. 3-MH is indicative of myofibrillar protein degradation and would not reflect an increase in cytosolic protein degradation. Nevertheless, after 4 wk of feed deprivation, MAFbx expression and 3-MH concentrations suggest an increase in cytosolic and myofibrillar protein degradation.

Treatment with IGF-I during feed deprivation inhibited the increase in MAFbx mRNA abundance and reduced expression of proteasome subunits A and R, which likely results in less protein degraded via ubiquitination and proteasome activity. The ability of IGF-I to inhibit upregulation of the proteasome system (21, 39, 59, 64) by signaling through the PI3K/Akt pathway to inactivate FOXO transcription factors and prevent upregulation of MAFbx expression (62) has been established in mammalian systems. It has been shown that IGF-I can stimulate the PI3K/Akt signal transduction pathway in trout myocytes (10), but the results presented here are the first to show effects of IGF-I on MAFbx expression in rainbow trout. Activation of ubiquitin ligases similar to MAFbx is essential for muscle atrophy (8); therefore, it is likely that the IGF-I-induced inhibition of MAFbx upregulation is a mechanism partially responsible for the reduced weight loss observed during feed deprivation in the present study. This idea is supported by the negative correlation between plasma IGF-I and *1*) percent wet weight loss, *2*) MAFbx expression, and

3) proteasome subunit R expression. In addition, a lower level of proteasome activity in IGF-I-treated fish than in saline-treated fish suggests that the protein-sparing actions of IGF-I are exerted throughout the entire ubiquitin-proteasome system.

The cathepsin-containing lysosomal pathway is a second route of protein degradation that was upregulated in white muscle during feed deprivation. This observation is consistent with previous results from fasted trout (57), rats (38), and salmon (46). In mammals, cathepsin activation during muscle atrophy occurs via a reduction of signaling through the PI3K/Akt signal transduction pathway, as described for the upregulation of the ubiquitin-proteasome system (81), which allows for a coordinated regulation of proteolysis. Thus the inhibition of cathepsin upregulation by IGF-I in the present experiment is consistent with this concept. In addition to nonselective degradation, lysosomes also contain a selective pathway of protein degradation that is activated during feed deprivation and functions to degrade proteins containing the KFERQ sequence motif (15), which is identified in ~30% of cytosolic proteins (16). Myofibrillar proteins, which make up 70–77% of protein in skeletal muscle, do not contain this sequence motif. In addition, inhibition of lysosomal function does not reduce myofibrillar degradation during muscle atrophy in rats (25, 41). If the same holds true for trout, the IGF-I-induced reduction of cathepsin expression may not be sparing muscle fiber but, rather, soluble cytosolic proteins.

The decrease in caspase-3 transcript observed in the present experiment is different from the observation of Nakashima et al. (48) in muscles from chicks fasted for 24 h. In contrast to salmonids, chicks are not accustomed to surviving extended periods of feed deprivation, so the ability of the trout to prevent upregulation of apoptosis in skeletal muscle during fasting likely enables preservation of muscle protein. The absence of widespread effects of IGF-I on caspase mRNA abundance suggests that the ability of IGF-I to reduce weight loss during short-term fasts is likely not the result of a reduction of apoptosis in white muscle. However, previous research in rainbow trout oocytes (80) and rodent muscle (18) and intestinal cells (20) suggests that IGF-I is able to inhibit apoptosis by decreasing caspase activation.

In the present experiment, calpain transcript abundance was decreased, whereas transcript abundance of the inhibitory calpastatins was increased with feed deprivation. The upregulation of calpastatins is consistent with previous research in rainbow trout (60). Although the decrease in calpain activity in the present experiment is consistent with the changes in transcript abundance, it contradicts previous observations in muscle from fasted trout (60). Results from previous research in rodents (21, 78) and rodent cell culture (31, 39) have been inconsistent with respect to the effects of IGF-I on calcium-dependent proteolysis by calpains.

The overall effects of feed deprivation and IGF-I on expression of genes within proteolytic pathways in rainbow trout white muscle are shown in Fig. 5. In summary, the feed deprivation-induced increase in MAFbx and cathepsin gene expression was ameliorated by IGF-I treatment, suggesting that the reduction in circulating IGF-I during feed deprivation affects proteolysis by upregulating the expression of these genes. Regulation of proteasome components and caspase-8 was also observed (Fig. 5), indicating an interaction of feed deprivation and IGF-I in expression of genes within multiple proteolytic pathways.

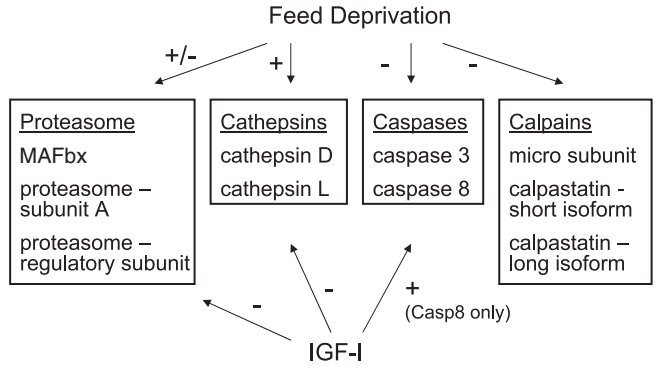

Fig. 5. Regulation of gene expression within proteolytic pathways by feed deprivation and IGF-I in rainbow trout white muscle. Symbols indicate direction of regulation: upregulation (+) and downregulation (−). MAFbx, muscle atrophy F-box/atrogin-1.

The family-dependent effects of saline and IGF-I treatment on wet weight loss did not translate into similar interactions in gene expression. In addition, the absence of an effect of IGF-I on protein loss suggests that the IGF-I weight loss response is not solely dependent on regulation of proteolytic gene expression. The effects of IGF-I on protein abundance, posttranscriptional modifications, or indexes of protein synthesis were not examined, and the effects of IGF-I on these mechanisms could certainly contribute to the observations regarding variables of weight loss.

Family averages in 28-day wet weight loss were correlated with changes in abundance for several proteolytic transcripts. After 14 and 28 days of feed deprivation, there were correlations between family means of changes in gene expression within and among proteolytic pathways. In addition, these correlations suggest that regulation of multiple genes within proteolytic pathways is coordinated in a way that supports a similar physiological response. These correlations imply that there is genetic variation in the physiological pathway (e.g., regulation of transcript abundance) connecting feed deprivation to proteolysis and weight loss. Correlations between individual values, but not family averages of plasma IGF-I and gene expression and weight loss, suggest that the genetic variation that results in differences in IGF-I concentrations may not be extended to similar changes in transcript abundance.

Perspective and Significance

In summary, this study demonstrates that the effects of feed deprivation on the proteasomal and lysosomal pathways in rainbow trout white muscle are similar to those in mammals, in that they are mediated by decreases in plasma IGF-I. In addition, the absence of a notable upregulation of calcium- and caspase-dependent proteolytic pathways may contribute to the ability of salmonid species to avoid the rapid weight loss associated with feed deprivation in warm-blooded animals. Family variations in gene expression that correlate with weight loss imply that genetic variation leading to differences in regulation of proteolytic genes serves as a mechanism contributing to family differences in fish weight loss in response to feed deprivation. These findings suggest that variations in rates and regulation of protein degradation may contribute to differences in growth performance among rainbow trout families. Further research into the mechanisms

responsible for the regulation of protein turnover is critical in rainbow trout *1*) for a better understanding of how nutrients are utilized and *2*) for the development of new strategies aimed at improving growth and nutrient utilization.

ACKNOWLEDGMENTS

We thank David Payne and Lisa Radler for technical assistance. We acknowledge the efforts of Jill Corbett, Cecilia Castano Sanchez, Juanita Engels, Jim Everson, Josh Kretzer, and Jenea McGowan.

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

GRANTS

This study was supported by Agricultural Research Service Project 1930-31000-007-00D.

REFERENCES

1. **Ando S, Hatano M.** Myofibrillar protein degradation in spawning-migrating chum salmon, as evaluated by extractive N^{τ}-methylhistidine level. *Bull Jpn Soc Sci Fish* 52: 1237–1241, 1986.
2. **AOAC.** *Official Methods of Analysis of AOAC International*. Gaithersburg, MD: Association of Analytical Communities, 2000.
3. **Asakawa K, Hizuka N, Takano K, Horikawa R, Sukegawa I, Demura H, Shizume K.** Effects of insulin-like growth factor I or human growth hormone in fasted rats. *Growth Regul* 2: 40–44, 1992.
4. **Ballard FJ, Tomas FM.** 3-Methylhistidine as a measure of skeletal muscle protein breakdown in human subjects: the case for its continued use. *Clin Sci (Lond)* 65: 209–215, 1983.
5. **Bark TH, McNurlan MA, Lang CH, Garlick PJ.** Increased protein synthesis after acute IGF-I or insulin infusion is localized to muscle in mice. *Am J Physiol Endocrinol Metab* 275: E118–E123, 1998.
6. **Beardall CH, Johnston IA.** Muscle atrophy during starvation in a marine teleost. *Eur J Cell Biol* 29: 209–217, 1983.
7. **Blaise O, Weil C, Le Bail PY.** Role of IGF-I in the control of GH secretion in rainbow trout (*Oncorhynchus mykiss*). *Growth Regul* 5: 142–150, 1995.
8. **Bodine SC, Latres E, Baumhueter S, Lai VK, Nunez L, Clarke BA, Poueymirou WT, Panaro FJ, Na E, Dharmarajan K, Pan ZQ, Valenzuela DM, DeChiara TM, Stitt TN, Yancopoulos GD, Glass DJ.** Identification of ubiquitin ligases required for skeletal muscle atrophy. *Science* 294: 1704–1708, 2001.
9. **Buonomo FC, Baile CA.** Influence of nutritional deprivation on insulin-like growth factor I, somatotropin, and metabolic hormones in swine. *J Anim Sci* 69: 755–760, 1991.
10. **Castillo J, Ammendrup-Johnsen I, Codina M, Navarro I, Gutierrez J.** IGF-I and insulin receptor signal transduction in trout muscle cells. *Am J Physiol Regul Integr Comp Physiol* 290: R1683–R1690, 2006.
11. **Castillo J, Codina M, Martinez ML, Navarro I, Gutierrez J.** Metabolic and mitogenic effects of IGF-I and insulin on muscle cells of rainbow trout. *Am J Physiol Regul Integr Comp Physiol* 286: R935–R941, 2004.
12. **Castillo J, Le Bail PY, Paboeuf G, Navarro I, Weil C, Fauconneau B, Gutierrez J.** IGF-I binding in primary culture of muscle cells of rainbow trout: changes during in vitro development. *Am J Physiol Regul Integr Comp Physiol* 283: R647–R652, 2002.
13. **Codina M, Garcia de la Serrana D, Sanchez-Gurmaches J, Montserrat N, Chistyakova O, Navarro I, Gutierrez J.** Metabolic and mitogenic effects of IGF-II in rainbow trout (*Oncorhynchus mykiss*) myocytes in culture and the role of IGF-II in the PI3K/Akt and MAPK signaling pathways. *Gen Comp Endocrinol* 157: 116–124, 2008.
14. **Davis TA, Fiorotto ML, Burrin DG, Vann RC, Reeds PJ, Nguyen HV, Beckett PR, Bush JA.** Acute IGF-I infusion stimulates protein synthesis in skeletal muscle and other tissues of neonatal pigs. *Am J Physiol Endocrinol Metab* 283: E638–E647, 2002.
15. **Dice JF, Chiang HL.** Lysosomal degradation of microinjected proteins. *Revis Biol Celular* 20: 13–33, 1989.
16. **Dice JF, Chiang HL.** Peptide signals for protein degradation within lysosomes. *Biochem Soc Symp* 55: 45–55, 1989.
17. **Dobly A, Martin SA, Blaney SC, Houlihan DF.** Protein growth rate in rainbow trout (*Oncorhynchus mykiss*) is negatively correlated to liver 20S proteasome activity. *Comp Biochem Physiol A Mol Integr Physiol* 137: 75–85, 2004.
18. **Dobrowolny G, Aucello M, Molinaro M, Musaro A.** Local expression of mIgf-1 modulates ubiquitin, caspase and CDK5 expression in skeletal muscle of an ALS mouse model. *Neurol Res* 30: 131–136, 2008.
19. **Dong H, Lin H, Jiao HC, Song ZG, Zhao JP, Jiang KJ.** Altered development and protein metabolism in skeletal muscles of broiler chickens (*Gallus gallus domesticus*) by corticosterone. *Comp Biochem Physiol A Mol Integr Physiol* 147: 189–195, 2007.
20. **Dube PE, Rowland KJ, Brubaker PL.** Glucagon-like peptide-2 activates β-catenin signaling in the mouse intestinal crypt: role of insulin-like growth factor-I. *Endocrinology* 149: 291–301, 2008.
21. **Fang CH, Li BG, Wray CJ, Hasselgren PO.** Insulin-like growth factor-I inhibits lysosomal and proteasome-dependent proteolysis in skeletal muscle after burn injury. *J Burn Care Rehabil* 23: 318–325, 2002.
22. **Franch HA, Price SR.** Molecular signaling pathways regulating muscle proteolysis during atrophy. *Curr Opin Clin Nutr Metab Care* 8: 271–275, 2005.
23. **Frost RA, Lang CH.** Differential effects of insulin-like growth factor I (IGF-I) and IGF-binding protein-1 on protein metabolism in human skeletal muscle cells. *Endocrinology* 140: 3962–3970, 1999.
24. **Fruhbeck G, Muguerza B, Castilla-Cortazar I, Santidrian S.** Effect of aging on the rate of muscle protein turnover in rat. *Rev Esp Fisiol* 52: 207–214, 1996.
25. **Furuno K, Goodman MN, Goldberg AL.** Role of different proteolytic systems in the degradation of muscle proteins during denervation atrophy. *J Biol Chem* 265: 8550–8557, 1990.
26. **Gabillard JC, Kamangar BB, Montserrat N.** Coordinated regulation of the GH/IGF system genes during refeeding in rainbow trout (*Oncorhynchus mykiss*). *J Endocrinol* 191: 15–24, 2006.
27. **Gallardo M, Castejon C, Navarro I, Blasco J, Gutierrez J, Sanchez J.** L-Leucine and L-alanine uptake by trout (*Salmo trutta*) cardiomyocytes: the effect of IGF-I and insulin. *Fish Physiol Biochem* 25: 239–248, 2001.
28. **Greene MW, Chen TT.** Quantitation of IGF-I, IGF-II, and multiple insulin receptor family member messenger RNAs during embryonic development in rainbow trout. *Mol Reprod Dev* 54: 348–361, 1999.
29. **Heger J, Patras P, Nitrayova S, Karcol J, Dolesova P.** Lysine maintenance requirement and efficiency of its utilisation in young pigs as estimated by comparative slaughter technique. *Arch Anim Nutr* 62: 182–192, 2008.
30. **Heymsfield SB, Stevens V, Noel R, McManus C, Smith J, Nixon D.** Biochemical composition of muscle in normal and semistarved human subjects: relevance to anthropometric measurements. *Am J Clin Nutr* 36: 131–142, 1982.
31. **Hong D, Forsberg NE.** Effects of serum and insulin-like growth factor I on protein degradation and protease gene expression in rat L8 myotubes. *J Anim Sci* 72: 2279–2288, 1994.
32. **Johansen KA, Overturf K.** Alterations in expression of genes associated with muscle metabolism and growth during nutritional restriction and refeeding in rainbow trout. *Comp Biochem Physiol B Biochem Mol Biol* 144: 119–127, 2006.
33. **Johnston IA.** Quantitative analysis of muscle breakdown during starvation in the marine flatfish *Pleuronectes platessa*. *Cell Tissue Res* 214: 369–386, 1981.
34. **Kelley KM, Schmidt KE, Berg L, Sak K, Galima MM, Gillespie C, Balogh L, Hawayek A, Reyes JA, Jamison M.** Comparative endocrinology of the insulin-like growth factor-binding protein. *J Endocrinol* 175: 3–18, 2002.
35. **Kieffer J, Tufts B.** Effects of food deprivation on white muscle energy reserves in rainbow trout (*Oncorhynchus mykiss*): the relationships with body size and temperature. *Fish Physiol Biochem* 19: 239–245, 1998.
36. **Kumamoto T, Fujimoto S, Ito T, Horinouchi H, Ueyama H, Tsuda T.** Proteasome expression in the skeletal muscles of patients with muscular dystrophy. *Acta Neuropathol (Berl)* 100: 595–602, 2000.
37. **Lankford SE, Weber G.** Association between plasma growth hormone, insulin-like growth factor-I, and cortisol with stress responsiveness and growth performance in a selective breeding program for rainbow trout. *N Am J Aquac* 68: 151–159, 2006.
38. **Lecker SH, Jagoe RT, Gilbert A, Gomes M, Baracos V, Bailey J, Price SR, Mitch WE, Goldberg AL.** Multiple types of skeletal muscle atrophy involve a common program of changes in gene expression. *FASEB J* 18: 39–51, 2004.
39. **Li BG, Hasselgren PO, Fang CH, Warden GD.** Insulin-like growth factor-I blocks dexamethasone-induced protein degradation in cultured myotubes by inhibiting multiple proteolytic pathways. *J Burn Care Rehabil* 25: 112–118, 2004.

40. **Loughna PT, Goldspink G.** The effects of starvation upon protein turnover in red and white myotomal muscle of rainbow trout, *Salmo gairdneri* Richardson. *J Fish Biol* 25: 223–230, 1984.
41. **Lowell BB, Ruderman NB, Goodman MN.** Evidence that lysosomes are not involved in the degradation of myofibrillar proteins in rat skeletal muscle. *Biochem J* 234: 237–240, 1986.
42. **Lyndon AR, Houlihan DF, Hall SJ.** The effect of short-term fasting and a single meal on protein synthesis and oxygen consumption in cod, *Gadus morhua*. *J Comp Physiol [B]* 162: 209–215, 1992.
43. **Martin SA, Blaney S, Bowman AS, Houlihan DF.** Ubiquitin-proteasome-dependent proteolysis in rainbow trout (*Oncorhynchus mykiss*): effect of food deprivation. *Pflügers Arch* 445: 257–266, 2002.
44. **McCormick SD, Kelley KM, Young G, Nishioka RS, Bern HA.** Stimulation of Coho salmon growth by insulin-like growth factor I. *Gen Comp Endocrinol* 86: 398–406, 1992.
45. **Mendez E, Smith A, Figueiredo-Garutti ML, Planas JV, Navarro I, Gutierrez J.** Receptors for insulin-like growth factor-I (IGF-I) predominate over insulin receptors in skeletal muscle throughout the life cycle of brown trout, *Salmo trutta*. *Gen Comp Endocrinol* 122: 148–157, 2001.
46. **Mommsen TP.** Salmon spawning migration and muscle protein metabolism: the August Krogh principle at work. *Comp Biochem Physiol B Biochem Mol Biol* 139: 383–400, 2004.
47. **Moriyama S, Swanson P, Nishii M, Takahashi A, Kawauchi H, Dickhoff WW, Plisetskaya EM.** Development of a homologous radioimmunoassay for Coho salmon insulin-like growth factor-I. *Gen Comp Endocrinol* 96: 149–161, 1994.
48. **Nakashima K, Komatsu T, Yamazaki M, Abe H.** Effects of fasting and refeeding on expression of proteolytic-related genes in skeletal muscle of chicks. *J Nutr Sci Vitaminol (Tokyo)* 51: 248–253, 2005.
49. **Negatu Z, Meier AH.** In vitro incorporation of [^{14}C]glycine into muscle protein of gulf killifish (*Fundulus grandis*) in response to insulin-like growth factor-I. *Gen Comp Endocrinol* 98: 193–201, 1995.
50. **Oddy VH, Owens PC.** Insulin-like growth factor I inhibits degradation and improves retention of protein in hindlimb muscle of lambs. *Am J Physiol Endocrinol Metab* 271: E973–E982, 1996.
51. **O'Sullivan U, Gluckman PD, Breier BH, Woodall S, Siddiqui RA, McCutcheon SN.** Insulin-like growth factor-1 (IGF-1) in mice reduces weight loss during starvation. *Endocrinology* 125: 2793–2794, 1989.
52. **Parrizas M, Plisetskaya EM, Planas J, Gutierrez J.** Abundant insulin-like growth factor-1 (IGF-1) receptor binding in fish skeletal muscle. *Gen Comp Endocrinol* 98: 16–25, 1995.
53. **Peragon J, Barroso JB, Garcia-Salguero L, Aranda F, de la Higuera M, Lupianez JA.** Selective changes in the protein-turnover rates and nature of growth induced in trout liver by long-term starvation followed by re-feeding. *Mol Cell Biochem* 201: 1–10, 1999.
54. **Peterson BC, Simpson PR, Cain KD, Hardy RW, Schelling GT, Ott TL.** Effects of administration of somatostatin-14 and immunoneutralization of somatostain on endocrine growth responses in rainbow trout (*Oncorhynchus mykiss*). *J Fish Biol* 63: 506–522, 2003.
55. **Pfaffl MW.** A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 29: e45, 2001.
56. **Pierce AL, Shimizu M, Beckman BR, Baker DM, Dickhoff WW.** Time course of the GH/IGF axis response to fasting and increased ration in Chinook salmon (*Oncorhynchus tshawytscha*). *Gen Comp Endocrinol* 140: 192–202, 2005.
57. **Rescan PY, Montfort J, Ralliere C, Le Cam A, Esquerre D, Hugot K.** Dynamic gene expression in fish muscle during recovery growth induced by a fasting-refeeding schedule. *BMC Genomics* 8: 438, 2007.
58. **Rios FS, Moraes G, Oba ET, Fernandes MN, Donatti L, Kalinin AL, Rantin FT.** Mobilization and recovery of energy stores in traira, *Hoplias malabaricus* Bloch (Teleostei, Erythrinidae) during long-term starvation and after re-feeding. *J Comp Physiol [B]* 176: 721–728, 2006.
59. **Sacheck JM, Ohtsuka A, McLary SC, Goldberg AL.** IGF-I stimulates muscle growth by suppressing protein breakdown and expression of atrophy-related ubiquitin ligases, atrogin-1 and MuRF1. *Am J Physiol Endocrinol Metab* 287: E591–E601, 2004.
60. **Salem M, Nath J, Rexroad CE, Killefer J, Yao J.** Identification and molecular characterization of the rainbow trout calpains (Capn1 and Capn2): their expression in muscle wasting during starvation. *Comp Biochem Physiol B Biochem Mol Biol* 140: 63–71, 2005.
61. **Salem M, Silverstein J, Rexroad CE, Yao J.** Effect of starvation on global gene expression and proteolysis in rainbow trout (*Oncorhynchus mykiss*). *BMC Genomics* 8: 328, 2007.
62. **Sandri M, Sandri C, Gilbert A, Skurk C, Calabria E, Picard A, Walsh K, Schiaffino S, Lecker SH, Goldberg AL.** Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell* 117: 399–412, 2004.
63. **Saunderson CL, Leslie S.** Muscle growth and protein degradation during early development in chicks of fast and slow growing strains. *Comp Biochem Physiol A Comp Physiol* 89: 333–337, 1988.
64. **Schulze PC, Fang J, Kassik KA, Gannon J, Cupesi M, MacGillivray C, Lee RT, Rosenthal N.** Transgenic overexpression of locally acting insulin-like growth factor-1 inhibits ubiquitin-mediated muscle atrophy in chronic left-ventricular dysfunction. *Circ Res* 97: 418–426, 2005.
65. **Seiliez I, Panserat S, Skiba-Cassy S, Fricot A, Vachot C, Kaushik S, Tesseraud S.** Feeding status regulates the polyubiquitination step of the ubiquitin-proteasome-dependent proteolysis in rainbow trout (*Oncorhynchus mykiss*) muscle. *J Nutr* 138: 487–491, 2008.
66. **Shepherd BS, Drennon K, Johnson J, Nichols JW, Playle RC, Singer TD, Vijayan MM.** Salinity acclimation affects the somatotropic axis in rainbow trout. *Am J Physiol Regul Integr Comp Physiol* 288: R1385–R1395, 2005.
67. **Shimizu M, Beckman BR, Hara A, Dickhoff WW.** Measurement of circulating salmon IGF binding protein-1: assay development, response to feeding ration and temperature, and relation to growth parameters. *J Endocrinol* 188: 101–110, 2006.
68. **Shimizu M, Swanson P, Dickhoff WW.** Free and protein-bound insulin-like growth factor-I (IGF-I) and IGF-binding proteins in plasma of Coho salmon, *Oncorhynchus kisutch*. *Gen Comp Endocrinol* 115: 398–405, 1999.
69. **Shimizu M, Swanson P, Fukada H, Hara A, Dickhoff WW.** Comparison of extraction methods and assay validation for salmon insulin-like growth factor-I using commercially available components. *Gen Comp Endocrinol* 119: 26–36, 2000.
70. **Silverstein JT, Hostuttler M, Blemings KP.** Strain differences in feed efficiency measured as residual feed intake in individually reared rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquac Res* 36: 704–711, 2005.
71. **Stinefelt BM, Eya JC, Semmens KJ, Blemings KP.** Effect of diet and strain on growth and performance in hybrid bluegill. *N Am J Aquac* 66: 312–318, 2004.
72. **Tcherkas YV, Kartsova LA, Krasnova IN.** Analysis of amino acids in human serum by isocratic reversed-phase high-performance liquid chromatography with electrochemical detection. *J Chromatogr A* 913: 303–308, 2001.
73. **Toyohara H, Ito K, Hori N, Touhata K, Kinoshita M, Kutoba S, Sato K, Ohtsuki K, Sakaguchi M.** Effect of steroid hormone administration on the breakdown of muscle proteins in ayu. *Fish Sci* 64: 419–422, 1998.
74. **van Eijk HM, Deutz NE, Wagenmakers AJ, Soeters PB.** 3-Methylhistidine determined in plasma by "high-performance" lipid chromatography. *Clin Chem* 36: 556–559, 1990.
75. **Vary TC, Jefferson LS, Kimball SR.** Role of eIF4E in stimulation of protein synthesis by IGF-I in perfused rat skeletal muscle. *Am J Physiol Endocrinol Metab* 278: E58–E64, 2000.
76. **Wassner SJ, Orloff S, Holliday MA.** Protein degradation in muscle: response to feeding and fasting in growing rats. *Am J Physiol Endocrinol Metab Gastrointest Physiol* 233: E119–E123, 1977.
77. **Wilkinson RJ, Porter M, Woolcott H, Longland R, Carragher JF.** Effects of aquaculture related stressors and nutritional restriction on circulating growth factors (GH, IGF-I and IGF-II) in Atlantic salmon and rainbow trout. *Comp Biochem Physiol A Mol Integr Physiol* 145: 214–224, 2006.
78. **Wingertzahn MA, Zdanowicz MM, Slonim AE.** Insulin-like growth factor-I and high protein diet decrease calpain-mediated proteolysis in murine muscular dystrophy. *Proc Soc Exp Biol Med* 218: 244–250, 1998.
79. **Wong AO, Zhou H, Jiang Y, Ko WK.** Feedback regulation of growth hormone synthesis and secretion in fish and the emerging concept of intrapituitary feedback loop. *Comp Biochem Physiol A Mol Integr Physiol* 144: 284–305, 2006.
80. **Wood AW, van der Kraak G.** Inhibition of apoptosis in vitellogenic ovarian follicles of rainbow trout (*Oncorhynchus mykiss*) by salmon gonadotropin, epidermal growth factor, and 17β-estradiol. *Mol Reprod Dev* 61: 511–518, 2002.
81. **Zhao J, Brault JJ, Schild A, Cao P, Sandri M, Schiaffino S, Lecker SH, Goldberg AL.** FoxO3 coordinately activates protein degradation by the autophagic/lysosomal and proteasomal pathways in atrophying muscle cells. *Cell Metab* 6: 472–483, 2007.
82. **Ziegler A, Sticher O.** Electrochemical, fluorescence, and UV detection for HPLC analysis of various cysteine derivatives. *J High Resolut Chromatogr Chromatogr Commun* 11: 639–646, 1988.